

A COMPARATIVE STUDY OF ROOT COVERAGE USING OrACELL™ VERSUS  
SUBEPITHELIAL CONNECTIVE TISSUE GRAFT

A Thesis

by

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## ABSTRACT

Gingival recession is defined as a mucogingival deformity that includes the apical displacement of the marginal soft tissues below the cemento-enamel junction, loss of attached gingiva, and exposure of root surfaces. The purpose of this randomized control clinical study was to compare outcomes of root coverage and clinical attachment levels between autograft and allograft in areas of facial gingival recession. Autogenous connective tissue graft (CTG) and decellularized human dermis (OrACELL™, LifeNet Health) were investigated.

Twenty-four non-smoking, healthy patients, with 2mm or greater of facial gingival recession at a minimum of one site that classified as a Miller Class I, II, or III recession defect were included in the study. Patients were randomly assigned to either control (CTG) or OrACELL™ (test) groups. Both test and control sites were treated with identical surgical techniques. The following clinical parameters were evaluated: vertical recession (VR), horizontal recession (HR), probing depth (PD), clinical attachment level (CAL), presence or absence of bleeding on probing (BOP), papillary height and width, and the width of the keratinized tissue (KT). Measurements were made at baseline, 3-, and 6-months.

All 24 patients completed the study, with 23 patients having Miller Class III defects. Eleven sites received CTG while 13 sites received OrACELL™. Baseline mean VR (CTG =  $3.27 \pm 0.68$ mm and OrACELL™ =  $3.50 \pm 0.89$ mm) and CAL (CTG =  $4.86 \pm 0.74$ mm and OrACELL™ =  $4.73 \pm 0.90$ mm) showed no significant difference between groups. At 6 months, mean VR (CTG =  $0.59 \pm 0.70$ mm and OrACELL™ =



1.19±1.07mm) significantly decreased in both groups, whereas CAL (CTG = 1.90±1.00mm and OrACELL™ = 2.42±1.17mm) significantly increased in both groups. Differences between group means were not statistically significant. There was a noted increase in KT in both the CTG and OrACELL groups, but it was not statistically significantly different at 6 months (CTG = 2.54±1.62mm and OrACELL™ = 2.50±1.58mm) from baseline (CTG = 1.95±1.80mm and OrACELL™ = 2.03±0.95mm).

Based on the results of this study, VR and CAL improved significantly in both the CTG and OrACELL™ groups from baseline to 6 months post-operatively. There were no significant differences between the CTG group or OrACELL™ group in VR or CAL over the course of the study. Miller Class III recession defects responded similarly with both CTG and OrACELL™.

## DEDICATION

I dedicate my thesis to my mother, Joan, who was instrumental and unwaveringly supportive throughout all of my academic pursuits.

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## NOMENCLATURE

ADM	AlloDerm® Regenerative Tissue Matrix
CEJ	Cemento-enamel junction
CTG	Connective tissue graft
FGG	Free gingival graft
GTR	Guided tissue regeneration
MGJ	Mucogingival junction
PDM	Puros Dermis
PEM	PerioDerm™

## CONTRIBUTORS AND FUNDING SOURCES

### **Contributors**

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The data analyzed for Chapter III was provided by Dr. Kontogiorgos of the Department of Prosthodontics. The student completed all other work conducted for the thesis independently.

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# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

### **Gingival Anatomy**

Gingiva surrounds teeth in the oral cavity of a normal adult. Gingiva provides cover to the teeth up to the cementoenamel junction (CEJ), which is the point of transition from crown to root, and cover to the alveolar bone that supports the teeth (1, 2). The gingiva functions to provide a barrier and/or seal around teeth to prevent microbial penetration or mechanical damage (1). There are three types of gingiva that are uniquely designed to function relating to their anatomical location: attached gingiva, marginal gingiva, and the gingival sulcus (1).

The attached gingiva is tightly bound to the periosteum and begins apically at the mucogingival junction (MCJ) and extends coronally to the marginal gingiva on the facial aspect of the maxilla as well as both the facial and lingual aspects of the mandible (1). The palatal attached tissue blends faultlessly with palatal mucosa. It is firm and immovable compared to the loose, stretchable mucosa that lies apical to the MCJ (1). The marginal gingiva, or unattached gingiva, lies coronal to the attached gingiva and is the gingival border that forms a collar around the teeth (1). The free gingival groove demarcates the attached gingiva from the marginal gingiva (2). The gingival sulcus is a shallow V-shaped crevice around a tooth. The bottom of the sulcus is comprised of junctional epithelium; one lateral wall is made up of tooth structure and the other is made up of oral sulcular epithelium. A periodontal probe serves to measure the depth of the gingival sulcus. The interdental gingiva, or gingival col, is pyramidal in shape and

located directly under the contact point between teeth. The interdental tissue is continuous from the facial to the palatal/lingual aspect.

### **Gingival Dimensions**

Although Riggs is often referred to as the father of periodontics, the first individual often credited for clinically calibrating and evaluating the human gingiva is Bowers. His 1963 article reported measurements of the range and average widths of the attached gingiva. Bowers reported that the range of attached gingiva in humans varied from 1 to 9 millimeters (mm) and additionally described the greatest width at the maxillary lateral incisor position, which averaged 5.2mm (3). Furthermore, Bowers reported the least attached gingival width at the mandibular first premolar, which averaged 2.0mm (3). The study also reported several noted trends regarding the anatomy of the attached gingiva. First, that both the maxilla and the mandible both average the most attached gingiva on the respective central and lateral teeth; moving posteriorly the width decreases over the canines and 1<sup>st</sup> premolars but then increases slightly over the 2<sup>nd</sup> premolars and molars (3). Secondly, the study suggested that generally the attached gingiva is broader in the maxilla when compared to the mandible (3).

In 1996, Eger evaluated gingival thickness perpendicular to each tooth at the base of the sulcus of each tooth. It was found in the maxilla that 2<sup>nd</sup> molars had the thickest tissue ( $1.25 \pm 0.50\text{mm}$ ) and canines had the thinnest gingiva ( $0.93 \pm 0.30\text{mm}$ ) (4). In the mandible, Eger found a similar trend in that the 2<sup>nd</sup> molars also averaged the thickest tissue ( $1.52 \pm 0.36\text{mm}$ ) and the canine ( $0.80 \pm 0.22\text{mm}$ ) averaged the thinnest gingiva as well (4). Goaslind (1977) also measured gingival thickness on humans and reported that the free gingiva averaged  $1.56 \pm 0.39\text{mm}$  (5). It was also found that the thickness of the

attached gingiva averaged  $1.25 \pm 0.42\text{mm}$ , therefore these findings averaged together showed a total mean gingival thickness of 1.41 mm (5).

Ainamo & Talari (1976) evaluated the role age plays on attached gingiva. By examining the relationship of the CEJ to the mucogingival junction (MCJ) and inferior border of the mandible in 40 dental students with panoramic radiographs, it was found that the distance of the MCJ to inferior border of the mandible remains unchanged over time (6). The distance from the MCJ to CEJ increased over time but recession didn't develop which showed that the width of attached gingiva increases over time (6). Ainamo and Talari attributed this to MCJ staying at a genetically predetermined location. This study also found no difference in attached gingiva based on sex (6).

Following up on the role age plays on gingiva, Tenenbaum (1986) evaluated mid-facial attached gingiva in 331 individuals from the deciduous to the permanent dentition. It was found that the mean width of attached gingiva was unaffected by the natural progression of the dentition, and keratinized tissue was not significantly different from deciduous to permanent dentitions (7). Also noted in this study was the band of attached gingiva being thickest over the central and lateral incisors, then diminishing posteriorly to the canine and first premolar, and lastly increasing in average thickness over the second premolar and first molar (7). Finally, geographical differences in tissue width were found to follow a similar trend in both maxilla and mandible, but the maxilla had a tendency to have more keratinized tissue than the mandible (7).

### **Gingival Recession**

Although the amount of attached gingiva may not change with age, the relationship of the gingival margin with the CEJ commonly will (8). When the gingival

margin migrates away from the CEJ and begins to expose root surface, it is defined as gingival recession (8). The recession measurement, CEJ to the gingival margin, is combined with the corresponding probing depth to determine the clinical attachment loss (CAL). Recession can result from four main categories of etiologic factors: 1) periodontal disease, 2) mechanical forces, 3) iatrogenic factors, and 4) anatomic factors (9).

Periodontal disease is a chronic inflammatory condition of the periodontal tissues to dental plaque (10). Unresolved periodontal lesions can result in loss of connective tissue, periodontal ligament attachment, and bone (11). There are various stages of inflammation and destruction, according to Page & Schroeder, which are clinically recognized with erythema, edema, periodontal pocket formation, bleeding on probing, and recession (11). Typically gingival recession results from a lack of alveolar bone at the site; however, it is also conjectured that if the band of connective tissue is reduced by inflammatory invasion of sulcular epithelium, this too could subsequently lead to gingival recession (12).

Mechanical factors predominate with overaggressive tooth brushing techniques in a “washboard” fashion that lead to erosion of cervical enamel and recession of the gingival margin (13, 14). Not only does aggressive brushing disrupt gingival tissue, but it also denudes the subsequently exposed root structure (13). Smokeless tobacco usage is also associated with recession. Monten *et al* found that snuff users averaged significantly higher rates of recession than non-users (15). Factitial injuries, such as chronic fingernail or pencil trauma to the gingiva, have also been reported in the literature as risk factors for recession and mucogingival issues (16). The correlation between occlusal discrepancies and recession has been investigated in animals and humans. Several animal studies have

shown that occlusal discrepancies are a risk factor for mucogingival issues including recession (17, 18). Many human studies, on the other hand, have failed to show a significant association between occlusal traumatism and recession (19, 20).

Recession resulting from iatrogenic factors can be grouped into restorative dental treatment and orthodontic movement of teeth. Location and design of restorative margins can influence the gingival margin. Subgingival and overbulked restorative margins, in addition to overaggressive packing of impression cord, can result in recession (21). In partially edentulous patients, poorly designed removable partial denture frameworks can create or exacerbate gingival recession (21, 22). Also, overextended denture acrylic on removable partial dentures can lead to localized recession defects (22). Orthodontic movement of teeth outside of the alveolar bony housing can cause a loss of the alveolar plate and ultimately bony dehiscences (23, 24). It has been observed that a lack of buccal bone on the tooth root can lead to gingival recession (23, 24).

Anatomic factors can be grouped into hard tissue and soft tissue related factors. Hard tissue factors are most commonly bony dehiscences, as previously mentioned, which tend to naturally develop from improper tooth eruption (12). When teeth erupt too far facially or lingually, the dehiscences created predispose patients to recession (12, 25). Soft tissue factors are most often associated with a narrow band of keratinized tissue (12, 25, 26). Minimal keratinized tissue can be stable when patients faithfully follow recommended periodontal maintenance schedules (25, 26). Conversely, patients who deviate or only loosely adhere to their maintenance schedule tend to have worse plaque control (26, 27). Inflammation results from poor plaque control and recession ensues

(27). High frenum pull has also been found to be highly associated with recession, especially in the mandibular anterior teeth (28).

Recession defects are classified according to defect morphology and the first to describe these defects was Sullivan & Atkins. Four categories were created that are based on width, with wide recession defined as more than 2mm, and depth, with deep defects defined as more than 3mm (29, 30). The four categories are as follows: 1) Shallow-Narrow, 2) Shallow-Wide, 3) Deep-Narrow, and 4) Deep-Wide (29, 30). Some seventeen years later Miller proposed a separate classification system to include bone loss and tooth position, along with extent of recession. Class I defects are defined as marginal tissue recession that doesn't extend beyond the mucogingival junction, with no loss of interdental bone or soft tissue and no tooth malpositioning (31). Class II defects are defined as marginal tissue recession that does extend beyond the mucogingival junction with no loss of interdental hard or soft tissue and no tooth malpositioning (31). 100% root coverage is possible with Class I and Class II defects. Class III defects involve marginal soft tissue recession extending to or beyond the mucogingival junction with interdental hard and soft tissue loss, or with malpositioned teeth (31). Only partial root coverage can be expected with Class III recession defects. Class IV defects involve marginal soft tissue recession extending to or beyond the mucogingival junction with severe interdental bone and soft tissue loss, or with malpositioned teeth (31). Grafting of Class IV defects is ill-advised as no root coverage can be expected.

Albandar & Kingman (1999) utilized the NHANES III database to evaluate the prevalence of gingival recession in American adults. In this study it was estimated that 23.8 million individuals have one or more tooth surfaces with 3 or more millimeters

(mm) of gingival recession (32). Albandar & Kingman also extracted from the data set that the prevalence, extent, and severity of gingival recession increased with age (32). It was also inferred that men had significant higher risk of developing gingival recession compared to women, as well as non-Hispanic Blacks when compared to Hispanics and Whites (32). Kassab found that 50 percent of people between the ages of 18 and 64 have gingival recession of at least 1mm (33). Furthermore, 88 percent of those over 65 have lost gingival attachment in at least one site, concluding that the trend for increasing recession appears to progress with age (33). It is likely that gingival recession will become an increasing concern for patients, given the aging and cosmetically-minded populations of Europe and the United States.

Gingival recession can be improved or corrected with surgical intervention via gingival grafting, with the primary aim of gingival grafting being to obtain root coverage. The main indications for root coverage procedures include esthetic demands, root sensitivity, preventing future progression of recession, and class V carious lesions (32, 34, 35). The corrective surgical procedures include lateral sliding flaps (36, 37), double papilla grafts (38), free gingival grafts (FGG) (39, 40), coronally positioned flaps (41, 42), coronal repositioning of a previously placed FGG (43, 44), connective tissue grafts (CTG) (45), guided tissue regeneration (46-48), and allografts (49). According to Miller, the ultimate goal of these procedures is root coverage that reestablishes both esthetics and function with a sulcus exhibiting no bleeding on probing and less than 2 mm probing depth (31).



## **Gingival Grafting**

The first described gingival surgical procedure to obtain root coverage was the laterally-positioned flap technique published by Grupe & Warren in 1956 (37). This technique was made popular through usage primarily in the mandibular anterior region on single-site defects. This technique involves removing the inflamed tissue on the buccal of the tooth to be addressed. An incision is made one papilla distal to the defect and extended into the alveolar mucosa, which becomes split-thickness in nature, until enough mobility of the flap is provided to allow the flap to cover the adjacent tooth with initial mucogingival defect and be sutured into place (37). The percent of root coverage ranges from 69% to 72% (9, 50). The disadvantages of this technique are that it is only effective in single tooth defects and can lead to recession at the donor site. Furthermore, the adjacent teeth need to have significant vestibular depth and an adequate band of keratinized tissue (9). On the other hand, when indicated, the advantages of this technique are that it doesn't require a second surgical site, can produce esthetic results, and can be completed relatively fast (9).

Ten years after introducing the laterally-positioned flap, Grupe (1966) made some amendments to his technique through what he titled the modified laterally-positioned flap (51). Recession at the flap donor site was a common sequela of the original laterally-positioned flap. Grupe aimed to avoid this issue ensuring that for a donor site to be utilized it would require an adequate band of keratinized tissue/attached gingiva (51). The modification consists of making a horizontal incision apical to the gingival sulcus at the donor flap. The original technique called for making this incision at the gingival margin, which permitted conservation of the marginal gingiva coronal to the flap. Therefore, only

half of the keratinized tissue is to be employed with the lateral flap, thus leaving half of the keratinized tissue as a protective collar at the margin around the donor site to avoid the possibility of developing recession (51). The principal issue with this technique is limited case application in which an adequate amount of attached gingiva is present at the donor site (9).

In 1968 Cohen & Ross developed an alternative to the laterally-positioned flap known as the double papilla flap (38). The technique calls for the removal of a “V” shaped wedge of tissue on the gingival margin of the tooth affected by recession. Vertical incisions into the underlying submucosa are made at the line angles that diverge apically. A horizontal incision is made at the interdental papilla. The two papillae are elevated in a split-thickness approach in which the papillae are dissected off of the periosteum, then rotated over the recipient site. A cutback incision may be necessary to negate any tension on the graft. The two flaps are then sutured together starting at the base and proceeding coronally. The sutured flaps are then placed at or slightly coronal to the CEJ with a suspensory suture. This technique leaves bare periosteum bilaterally interproximally adjacent to the defect site but, unlike the lateral sliding flap, doesn’t remove the marginal keratinized tissue from adjacent teeth and is able to utilize primarily interdental tissue.

Further advantages of the double papilla technique are that there is additional blood supply from two separate flaps and less tension on the sutured flap (9). Disadvantages of this technique are that predictability of root coverage is relatively poor and there is a steep learning curve as the procedure is technique sensitive (9). Ross *et al* completed a follow up study in 1986 which evaluated 14 years of retrospective data (52).

It was concluded that the presence of wide and thick interdental papillae were advantageous and produced improved long term results (52).

Pennel & King were the first to introduce the Free Gingival Graft to the United States in 1964, however the first modern periodontist to introduce this technique to the world was Bjorn of Sweden in 1963 (53-55). The purpose of the free gingival graft initially was to increase the band of keratinized tissue and extend vestibular depth. Over time, the free gingival graft became more widely used for root coverage. Miller's classification system of recession defects is credited for this paradigm shift (31).

Sullivan and Atkins (1968) described the successful principles for free autogenous gingival grafting (29, 30). Briefly, the recipient site is denuded down to the periosteum through removal of the epithelium, connective tissue, and any muscular attachment, resulting in a bleeding bed created in anticipation of the donor graft. Although donor tissue can come from the maxillary tuberosity or edentulous ridges, it most commonly is obtained from the palate. The graft harvested from the palate includes the epithelium and a portion of the lamina propria. Donor grafts are defined as split-thickness or full-thickness grafts. Full-thickness grafts include the epithelium and the entire lamina propria and range in thickness from 1.25mm to 1.75mm. Split-thickness grafts are further labeled as intermediate or thick, with intermediate ranging from 0.5mm to 0.75mm and thick grafts ranging from 0.75mm to 1.25mm.

Free gingival graft dimensions need to be calculated based on the requirements of the recipient site, and the graft needs to be slightly oversized to account for graft shrinkage. It has been established that the thicker the graft, the greater the degree of primary graft contraction. Furthermore, the thinner the graft, the more secondary graft

contraction occurs. Mormann evaluated the effect of thickness of free gingival grafts over 12 months. He concluded that shrinkage was 45% for very thin grafts, 44% for thin grafts, 38% for intermediate, and 30% for grafts taken with a scalpel (56). Several studies have delved deeper into the percent shrinkage of free gingival grafts. Ward reported that over 6 months he witnessed 47% shrinkage in apico-coronal width of keratinized tissue at the graft site, Soehren *et al* witnessed 30% shrinkage over 7 months, and with 4 years of follow-up Rateitschak calculated 25% shrinkage all in an apico-coronal dimension (57-59). Rossmann & Rees reported that the graft area of a free gingival graft shrunk an average of 24% over 10 months (60). Furthermore, James & McFall compared shrinkage of free gingival grafts on a denuded bone bed vs. grafts on a periosteum covered bone bed. With 6-month data, they reported that grafts placed on a periosteal bed had 1.5 to 2 times more shrinkage than grafts placed on denuded bone (61). Dordick found that free gingival grafts placed on bone showed no mobility, had less swelling, and experienced better hemostasis when compared to grafts placed on periosteal beds (62). However, Dordick also noted that grafts placed on denuded bone expressed delayed healing for the first two weeks post-operatively (62).

Epithelium is removed with the graft from the donor site, which creates an open wound. The residual donor site often has a hemostatic agent introduced and is then covered with a surgical stent in an effort to aid in clot formation and ultimately hemostasis. The graft is then transferred to the recipient site and sutured into place. Strap and compression sutures are often utilized to improve graft adaptation to the recipient bed. This technique has several advantages, namely it's time-efficient, it can be extended to cover several teeth with recession defects, it isn't dependent on vestibular depth, and it

doesn't require any adjacent donor tissue (9). The disadvantages include bleeding from the donor site, pain, and a common esthetic mismatch or poor blending of tissue at the graft margins in the recipient site (9). The graft tends to appear lighter in color than the adjacent gingiva. Several studies have shown that a range of 70-90% root coverage can be achieved with a free gingival graft, with the average root coverage tallying roughly 88% (31, 39, 63).

Sullivan and Atkins famously published the process of free gingival graft incorporation at recipient sites (29, 30). The first designated stage is plasmatic circulation in which the graft is solely dependent upon diffusion from the surrounding tissue of the host bed and the fibrin clot. This stage takes place from Day 0 to Day 2. The next stage is revascularization, which is characterized by extension and growth of capillaries into the graft. This second stage takes place between Day 2 and Day 8. Capillaries start to infiltrate the graft on Day 1 but the graft cannot survive solely on capillary circulation usually until Day 8. Sullivan and Atkins went on to describe a third stage that was termed organic union. Connective tissue union between the graft and the recipient bed defines this stage. The organic union phase often begins at Day 4 and ends at Day 10.

Bernimoulin introduced to the periodontal community the coronally advanced flap in 1975. Various techniques have developed over the years but Bernimoulin was the first to present this technique with vertical incisions made at the line angles of defects with full-thickness flap reflection, and advancement with a partial-thickness periosteal release (44). The flap is then elevated coronally and sutured at the CEJ. Bernimoulin advocated measuring recession, then measuring the papilla, and creating new papilla tips that are measured apical to the original tips to equal the amount of recession. Once the

tissue has been reflected, the remaining papilla tips are de-epithelialized, and the flap is coronally positioned over the de-epithelialized papillae to cover the recession. One study reports mean root coverage of 97.8% at six months (41).

Tarnow introduced a different coronally advanced flap approach with the semilunar coronally positioned flap (42). The same requirements of the coronally positioned flap were maintained with this technique. A submarginal semilunar incision is placed on bone that follows the curvature and anatomy of the gingival margin. A sulcular incision is made as a second incision, which connects with the base of the flap that collectively is split-thickness in nature. This flap is coronally advanced, leaving periosteum exposed. No sutures are required for this technique; the blood clot formed from gentle pressure is enough to stabilize the gingival margin. This technique is not recommended for sites that are deficient in keratinized tissue. If inadequate keratinized tissue is present, a free gingival graft would need to be placed at the site and allowed to heal for at least 2 months before attempting a semilunar flap or any coronal advancement procedure (43, 44).

In 2000, Zucchelli & De Sanctis published a new technique that was designed to account for varying degrees of recession on adjacent teeth and to improve esthetic outcomes (64). Bernimoulin's technique commonly left evident scarring in the new papillae after coronal advancement and mucosal scarring as a result of vertical incisions necessary for flap advancement. Tarnow's semilunar incisions commonly caused scarring also in the regions of exposed periosteum that were created by the advancement of the gingival margin. Zucchelli pioneered the envelope flap, which lacked vertical incisions, and created new surgical papillae. A new surgical papilla is generated by an oblique

incision at the base of an existing papilla. The point of the papilla is at the distal of the interproximal space for the mesial half of the flap and on the mesial of the interproximal space for the distal half of the flap. The flap is a split-full-split design and the incision is extended an extra one tooth mesial and distal to the teeth to be covered. Closure is achieved with sling sutures. After 12 months of follow-up, Zucchelli & De Sanctis using their modified coronally advanced flap reported a 2.7mm reduction in recession and complete root coverage was achieved at 88.6% of sites (64). This technique and altered forms of this technique are still very popular in soft tissue grafting today.

While each procedure has its indication and place as it relates to soft tissue augmentation, the subepithelial connective tissue graft (CTG) is considered to be the best option in attaining root coverage (65). Langer & Langer first described the surgical technique in detail for successful connective tissue grafting for root coverage in 1985 (45). The coronal flap margin is started with a horizontal sulcular incision to preserve all existing radicular gingiva while leaving the interproximal papillae intact. Vertical incisions are made on both sides of the recession defect to include the interproximal papillae, and the split-thickness flap is reflected. At the palatal donor site a horizontal incision is made approximately 5-6mm from the gingival margins of the maxillary teeth to the desired width. It is continued apically as an inverse bevel towards alveolar bone. A second parallel incision is made 1.5- 2mm coronal to the first incision, and this incision is continued apically until it meets the base of the original incision. The palatal bone is scored to enable the operator to remove the connective tissue wedge. Vertical incisions are made on either side as needed to facilitate tissue removal. The palatal flap is then sutured back to its original position. The donor connective tissue and epithelium are

sutured to the underlying connective tissue interproximally to cover the denuded roots. The recipient flap is positioned coronally to cover as much of the graft as possible and sutured in this position. No attempt is made to completely cover the graft. Langer & Langer concluded that the use of the subepithelial connective tissue graft offers a combination of both pedicle flap and free gingival graft.

Raetzke published a new technique, also in 1985, utilizing the connective tissue graft with his envelope technique (66). The affected tooth has its gingiva undermined through the sulcus with a partial thickness incision that is used to create an envelope around the denuded root surface. A connective tissue graft is then harvested from the palate with two anterior-posterior incisions made 1-2mm apart. The incisions are angled in a similar fashion and should converge just short of the bone. A wedge of tissue is removed and the small band of epithelium is excised. The graft should be double the width of the area of recession. The graft is then placed in the envelope to cover the denuded root. Finger pressure and tissue adhesive are all that is required for the securing the graft; however, sutures are needed for the donor site. Raetzke found an average of 80% root coverage with this technique and a gain of 2.5-5mm of keratinized tissue (66).

With the popularity of the connective tissue graft on the rise, in 1992 Harris proposed the use of a connective tissue graft with a double pedicle flap (67). Vertical incisions are made perpendicular to the horizontal incisions through the papillae, starting at the termination point of the horizontal incisions and extending apically to the alveolar mucosa. A sulcular incision connects the horizontal incisions. Partial-thickness pedicle flaps are reflected, and the mesial and distal pedicle flaps are placed over the defect and sutured together with gut suture material. A connective tissue graft is then harvested from



the palate and placed over a denuded and flattened root surface and sutured with gut sutures to the interproximal papillae. The adjoined pedicle flaps are then sutured over the connective tissue with a sling suture. At 3 months 97.2% mean root coverage was achieved with 100% root coverage taking place 80% of the time (67). Furthermore, width of keratinized tissue increased from an average of 1.6mm to 4.8mm during this time (67).

In 1994 Allen published a paradigm-shifting technique that he titled the suprapariosteal envelope, and this technique came to be known as the “tunnel technique” (68). Allen proposed that the advantage of this technique was that incisions were minimal and there were no incisions through papillae, only undermining or tunnelization to maintain tissue integrity. Partial-thickness intrasulcular incisions are made over radicular areas that are to be treated. Incisions are extended 3-5mm apically and laterally to undermine interproximal papillae. A properly sized connective tissue graft is harvested from the palate and threaded through the tunnel created by the envelope flap. A portion of the graft will be apical to the flap and completely covered by intact interdental tissue, which is continuous with the papillae, and another portion of the graft will overlie the radicular surface. The graft is then tacked into place with sutures. This allows for the tunneled papillae to mechanically support the graft and provide less interruption of vasculature in the flap as a whole.

The tunnel technique was effective in single sites as shown by Allen, but Zabalegui illustrated that tunneling could also be successfully utilized for long spans of adjacent teeth that require root coverage (69). Zabalegui grafted multiple adjacent sites with palatally harvested connective tissue and after 12 months found an average reduction of 3.05mm of recession which averaged out to 92% root coverage (69).

Complete root coverage was achieved at 67% of the sites (69). Furthermore, Zuhr compared the tunnel technique to coronally advanced flaps (70). Connective tissue grafts were utilized for both groups in combination with enamel matrix derivative. After 12 months 98.4% root coverage was achieved with the tunnel technique while 71.8% was achieved with the coronally advanced flap.

Harris performed a split thickness flap in which he placed a connective tissue graft which was covered with a double pedicle flap (71). The teeth were scheduled for extraction due to prosthetic reasons, and a biopsy was taken with the extractions at 6 months healing to evaluate connective tissue graft attachment. Two different healing patterns were observed: (1) long junctional epithelial attachment with minimal connective tissue adjacent to tooth, and (2) short junctional epithelium that stopped at the previously exposed root. The latter pattern expressed very minimal epithelial attachment, but rather predominately connective tissue attachment. Most importantly, no new bone or cementum was visualized in any section and therefore true regeneration was not observed in this case report (71). Majzoub evaluated connective tissue grafts after 12 months of healing histologically and also found healing primarily occurred via long junctional epithelium (72). Minimal signs of new cementum-like tissue formation were noted in the apical portion of the recession area.

Although Harris and Majzoub did not observe true regeneration in their respective studies, other case reports have shown evidence of regeneration. Bruno & Bowers evaluated attachment after connective tissue grafting of long standing facial recession (73). Multiple types of attachment were noted histologically, which included some periodontal regeneration. It was noted that the greatest area of exposed root surface

covered by connective tissue adhesion was at 1 year. Goldstein also histologically evaluated a single extracted tooth 6 months after connective tissue grafting for root coverage (74). New connective tissue attachment was observed which included new periodontal ligament. Therefore, from the literature, periodontal regeneration has been shown to occur via connective tissue grafting for root coverage.

A potential added benefit of tissue grafting for root coverage is “creeping attachment.” Goldman (1964) describes this phenomenon as a post-operative migration of the free gingival margin (75). Matter observed that this phenomenon took place 1 month to 1 year after free gingival grafting with no marginal changes observed between 1 and 5 years (76). After performing connective tissue grafts with double pedicle flaps, Harris observed an average creeping attachment of 0.8mm at 12 months (77). Furthermore, this creeping attachment was reported to have occurred at 96% of sites.

Guided tissue regeneration (GTR) is the term used to describe a class of procedures designed to regenerate lost periodontal structures that include bone, cementum, and periodontal ligament. These procedures utilize barrier membranes to cover defects to exclude epithelium (78). Regeneration of lost periodontal tissue has been attempted around recession defects using a combination of resorbable and non-resorbable barrier membranes (46-48). The early studies by Tinti involved isolating a defect, placing a non-resorbable membrane over the defect, and then coronally advancing a flap over the surface of the membrane (46). After removing the non-resorbable membranes at 4 weeks and 6 months post-operatively, the results illustrated significant differences in recession reduction, to include a significant gain in attachment. In an effort to avoid a second stage surgery for membrane removal, Pini-Prato integrated a similar approach but utilized

resorbable membranes composed of polygalactic acid and citric acid esters (Guidor™) rather than non-resorbable membranes (47). Similarly, significant improvements were noted for recession coverage and clinical attachment gain at 6 months post-operatively compared to baseline.

Focusing on gingival recession, Rocuzzo compared efficacy of both resorbable and non-resorbable membranes. He noted significant improvements in probing depth reduction and clinical attachment gain between baseline and at 6 months post-operatively. Root coverage, however, was comparable for both groups. Non-resorbable membranes achieved 83.2% coverage while resorbable membranes achieved 82.4% coverage, which was determined to not be statistically different (48).

Long-term stability of surgical treatment of recession with GTR was evaluated by Pini-Prato utilizing non-resorbable membranes (79). Four-year follow-up was utilized to investigate root coverage. The height of the gingival margin was deemed stable, as there was no significant change in root coverage. It was also concluded that GTR with non-resorbable membranes significantly increased the amount of keratinized tissue. This was attributed to apical migration of the mucogingival junction over time. Harris re-evaluated GTR cases treated with resorbable membranes at an average of 25.3 months post-operatively (80). Contrary to Pini-Prato, Harris noted a significant regression in root coverage over time. He found 92.3% coverage at six months, which decreased to 58.8% at two years. This indicated that GTR may not be the treatment of choice long term in achieving root coverage.

Many articles, when addressing success of grafting, reference root coverage by defining it as the portion of the root covered by tissue post-operatively. Other authors

evaluate defect coverage by defining it as the portion of the defect covered by tissue post-operatively. In an effort to correct the lack of standardization in GTR and soft tissue grafted cases, Greenwell suggested that the success of root coverage procedures should be based on a mean root length of 13.6mm. Greenwell claims this would allow for simultaneous evaluation of both defect coverage and defect elimination of a particular surgical technique (81). Under this pretense, a 1mm defect would always result in 93% coverage and a 5mm defect would result in 63% root coverage (81).

### **Allografts**

Connective tissue grafting has been deemed the gold standard for soft tissue grafting for root coverage (65). The biggest drawback of performing connective tissue grafting procedures is the requirement of a second surgical site, the palate. The site being treated plus the palatal harvest site increase morbidity of this root coverage procedure compared to a pedicle flap or coronally advanced flap where no palatal harvest is necessary. Another drawback is the finite amount of tissue available for harvesting on the palate. In patients that require treatment on multiple teeth in multiple quadrants, there simply isn't enough tissue available to achieve root coverage without several surgical procedures spaced out to allow for palatal healing and palatal connective tissue regeneration. The desire to not be limited in the amount of teeth that can be treated at one time coupled with the desire to decrease post-operative morbidity by doing away with the palatal harvest has led to development of the acellular dermal matrix.

Acellular dermal matrix (ADM) is human dermis tissue that has been processed to remove both epithelium (epidermis) and cells that can lead to tissue rejection and graft failure, without damaging the matrix. The processed tissue is preserved with a patented

freeze-drying process that prevents damaging ice crystals from forming. Of the current products on the market, AlloDerm™ Regenerative Tissue Matrix (BioHorizons, Birmingham, AL) is the longest used and most studied (82). The ADM tissues are obtained from independent third-party American Association of Tissue Banks (AATB) guideline-compliant facilities. Tissues are transported in culture media containing various concentrations of gentamicin, cefoxitin, lincomycin, polymyxin B, and vancomycin (83). Prior to tissue acceptance, donor blood is tested for bloodborne diseases such as hepatitis B, hepatitis C, and HIV as well as pathogenic bacteria (82).

There is a 3-step proprietary process that is utilized to prepare the tissue. The process begins with a high-ionic strength solution that serves to separate the dermis from the epidermis while leaving the basement membrane intact through intercellular bonds between layers (83-85). The purpose of secondary washing is to remove major histocompatibility class I and II antigens from the donor tissue using sodium deoxycholate (83, 85, 86). The final step involves the creation of an amorphous ice through a freeze-drying process that allows for the intricate microarchitecture of the dermis to retain its structural integrity (85). After the process is completed, undamaged fibers and bundles of type I, III, IV and VII collagen, laminin, and elastin are all that remain alongside the glycosaminoglycans (83-85, 87).

The ADM allograft arrives sealed within two Tyvek backs, containing trace amounts of antibiotics (88). The tissue must be rehydrated in two 0.9% normal saline baths, for a total rehydration time of 10 to 40 minutes. The final tissue thickness ranges from 0.9mm to 1.6mm, and once placed, the matrix acts as a scaffold (89). The allograft

promotes in-growth of fibroblasts and endothelial cells along the porous dermal aspect and also epithelial migration along the basal lamina (83, 90).

Clinically, no significant differences have been brought to light regarding recession coverage, keratinized tissue, probing depth, and clinical attachment levels between connective tissue grafts and ADM (91). It has been noted that the gingival attachment is similar histologically, with long junctional epithelium and connective tissue adhesion occurring in both grafts (92). Both grafts have been found to incorporate well within recipient tissues, with new fibroblasts, endothelial cells, and collagen distributed uniformly throughout sites. The only notable difference histologically is that ADM has more elastin fibers present than the connective tissue graft (92, 93).

Another commercially available acellular dermal allograft material is Puros Dermis™ (PDM) (Zimmer Dental, Carlsbad, CA). PDM, like Alloderm™, has been marketed as a substitute for autogenous soft tissue grafting. Zimmer maintains that PDM can be used for both root coverage and horizontal soft tissue augmentation (94). PDM is processed with Zimmer's proprietary method known as the Tutoplast® Process. This process is a multi-stage method that employs solvent-dehydration in conjunction with gamma irradiation followed by freeze-drying of the graft (95). The preliminary phase integrates an osmotic treatment to kill bacteria and reduce viral load (94). The second stage utilizes an oxidative treatment to destroy remaining proteins in an effort to minimize the possibility of graft rejection. The function of the solvent-dehydration step is to remove water while further disinfecting the matrix also reducing prions. In an effort to provide a  $10^{-6}$  level of sterility, the final sterilization step incorporates limited-dose gamma irradiation. Like Alloderm™, the tissue is delivered in a double-sealed package;

however, unlike ADM, PDM is reported to rehydrate in 30 seconds but can be rehydrated for up to 30 minutes to improve handling properties (96, 97). PDM has a 5 year shelf-life and can be stored at room temperature. The final tissue thickness of PDM can range from 0.8mm to 1.8mm (94).

Several studies have clinically evaluated PDM in root coverage procedures. A multicenter randomized control trial was performed comparing ADM and PDM with coronally advanced flaps over 52 weeks on Miller Class I and II defects (98). Both groups showed a statistically significant benefit to root coverage with an average of 77% root coverage for the ADM and 71% root coverage for the PDM. A significant difference was not noted between the two materials. Another randomized control trial was completed utilizing split-mouth design also comparing root coverage for ADM and PDM but with Miller Class I and III defects (97). This study concluded the average percent root coverage for ADM and PDM to be 83.4% and 81.4% respectively, the difference determined to not be statistically significant.

Another recently launched acellular dermal allograft is PerioDerm™ (PEM) (Dentsply Implants, Waltham, MA). PerioDerm™ has also been recommended for root coverage and horizontal soft tissue augmentation for soft tissue defects (99). The sterilization of PEM is done through the Musculoskeletal Transplant Foundation, which exceeds the standard of the AATB. Prior to being selected for tissue harvesting, the donors' blood is screened for hepatitis B, hepatitis C, HIV, and syphilis. Donors are further screened for cancer and illicit drug usage. Dentsply's processing goal for PerioDerm™ is to maintain the integrity of the extracellular matrix so the tissue is minimally processed only to remove epidermal and dermal cells. Like PDM, PEM also



has a three-phase proprietary sterilization method. The first phase utilizes sodium chloride to remove the epidermal and dermal cells from the donor tissue. The second phase employs a Triton™ X-100 detergent wash that removes any and all remaining cellular debris. The third phase includes propriety disinfection and freeze-drying that is able to reduce bacteria and fungi to  $10^{-6}$  and the viral load from  $10^{-4}$  to  $10^{-6}$ . What makes this process unique is no sensitizing agents or  $\beta$ -lactam antibiotics are used in processing (100). Biologic integrity is maximized via this proprietary process, which avoids high-dose gamma irradiation. PEM is also packaged in two separate Tyvek bags. PEM requires 3 to 5 minutes of rehydration in sterile 0.9% normal saline or lactated Ringer's solution, which is shorter than ADM but slightly longer than PDM. PEM has no need for refrigeration and a 3 year shelf-life. The final tissue thickness of PEM ranges from 0.8mm to 1.7mm, and each piece is quality-controlled for 90%+ uniformity in thickness. At the present time there are no peer-reviewed articles regarding clinical application of PEM. However, a non-controlled case study is available through the distributor's website (99).

### **Root Modification**

When attempting to achieve root coverage, several different types of root biomodification have been suggested to enhance soft tissue attachment to the exposed root surface (101). Materials utilized are expected to function by exposing new collagen fibers through the removal of the smear layer and any potential pathogens that may prevent fibroblast adhesion to root structure (102). The original modifier utilized was citric acid. Bertrand compared clinical outcomes of 20 free gingival grafts in a split-mouth study with citric acid root biomodification vs. no biomodification (103). He found

that citric acid, which has a pH of 1, didn't contribute any significant benefit to the clinical outcome. Furthermore, Caffesse evaluated the effect of citric acid in a randomized control trial that compared connective tissues with and without citric acid root biomodification on 36 recession sites (104). He concluded that the root demineralization resulting from citric acid application did not have any effect on clinical outcomes.

Ethylenediaminetetraacetic acid (EDTA, PrefGel™) is another material that is commonly used as a root biomodifier. EDTA is a chelating agent that removes inorganic debris and the smear layer. Kassab evaluated root coverage with connective tissue grafts on 20 teeth in 10 patients in a split-mouth study comparing 2 minutes of root preparation with 24% EDTA vs. sterile saline (105). He found that EDTA did not provide any significant benefit to total root coverage. Furthermore, in 2007 Bittencourt used a semilunar coronally positioned flap with or without EDTA to cover recession defects (106). Root coverage was significantly higher in the defects not treated with EDTA and so concluded that use of EDTA negatively affected root coverage (106). In a similarly constructed study Nd:YAG lasers were utilized for root preparation during root coverage procedures and also were found not to enhance root coverage and possibly negatively impact coverage (107).

A recent systematic review delved into root surface biomodification and concluded that use of any chemical agent has not been statistically proven to benefit any clinical parameters that have been measured (108). Ultimately, the bulk of the literature has shown no evidence to support the use of root surface biomodifiers prior to root coverage treatment.

## CHAPTER II

### STUDY DESIGN

#### **Background to Issue**

Gingival recession is a condition that affects an extensive range and percentage of people. In an evaluation of epidemiological data, Kassab found that 50 percent of people between the ages of 18 to 64 have gingival recession of at least 1mm (33). Furthermore, 88 percent of those over 65 have lost gingival attachment on at least one site, concluding that the trend for increasing recession appears to progress with age. It is likely that gingival recession will become an increasing concern for patients, given the aging and cosmetically-minded populations of Europe and the United States (109).

The main indications for root coverage procedures include esthetic demands, root sensitivity, preventing future progression of recession, and treating class V carious lesions (31). According to Miller, the ultimate goal of these procedures is root coverage that reestablishes both esthetics and function with a sulcus exhibiting no bleeding on probing and less than 2mm probing depth (110).

Numerous techniques have been used to treat gingival recession. The current gold standard for root coverage is the subepithelial connective tissue graft (CTG) technique, which requires a connective tissue graft harvested from the palate and placed over an appropriate recession defect with coronal advancement of flap over graft (45). This procedure, therefore, requires a donor and recipient site leading to greater patient discomfort and increased surgical time (31). However, given the reluctance of patients to tolerate additional surgical sites and the necessity to obtain large tissue sections for full mouth treatment, periodontists have turned to allograft substitutes.

Allograft materials for gingival recession treatment have become mainstays in the periodontal field. It was demonstrated in a study by Harris that acellular dermal allograft materials (ADM) like AlloDerm™ are viable substitutes for CTG in the treatment of gingival recession defects (31).

AlloDerm™ material is human dermis processed to remove the epidermal layer along with all of the dermal cellular structures, thereby removing factors responsible for graft rejection and infection. This allograft then acts as a scaffold for the fibroblasts to repopulate the connective tissue matrix and encourages epithelial cells to migrate from the adjacent tissue margins.

Within the current body of literature, support of the efficacy of AlloDerm™ is abundant. Tailing the success of AlloDerm™ for a range of procedures including: (1) increasing keratinized tissue width, (2) root coverage, (3) guided tissue regeneration, and (4) guided bone regeneration, new decellularized dermis products have infiltrated the market. In contrast to AlloDerm™, very little research has been conducted on the efficacy of decellularized dermis.

The manufacturer of a decellularized dermis material, LifeNet Health, says that OrACELL™ retains native growth factors along with collagen and elastin (111).

Therefore, OrACELL™ is potentially an excellent candidate for guided tissue regeneration, guided bone regeneration, and oral soft tissue correction (111).

The proposed study is designed to compare the differences in clinical parameters of root coverage surgeries using OrACELL™ versus CTG.

## **Materials and Methods**

This study was approved by the Texas A&M University College of Dentistry Institutional Review Board (IRB) and undertaken as a randomized, prospective clinical trial. Informed consent documentation was approved by the IRB for use in this study.

A power analysis (G\*Power 3.1.2) was performed before initiating the study. A two-tailed t-test determined that 15 surgical sites for test and control (total of 30 sites) would be required to determine a difference of 0.5 mm between groups ( $\alpha = 0.05$ ,  $1 - \beta = 0.8$ ).

### ***Patient Population***

Non-smoking, healthy patients were chosen for the study based on their existing recession. The inclusion criteria were as follows: (1) patients must be between 18 and 80 years old, with recession present in one quadrant as either single or multiple buccal vertical recession sites; (2) the defect must be at least 2mm in length, (measured from the CEJ to the midfacial gingival margin) and classified as either Miller Class I, II or III (31); and (3) study is limited to vital and nonvital incisors, canines, and premolars. If teeth adjacent to the site to be treated had recession as well, they were included in the grafting procedure but not included in measurement. Plaque control defined as modified O'Leary Index of 85% or more after initial therapy was established before surgical intervention (112). Only sites with probing depths of 3mm or less and no bleeding on probing were accepted for surgery.

The exclusion criteria were as follows: (1) subjects who smoke more than ten cigarettes per day or use nicotine replacement therapy; (2) previous history of surgery performed at surgical sites included in study; (3) subjects who have uncontrolled or

poorly controlled systemic conditions that could compromise or contraindicate periodontal surgery; (4) non-English speakers; (5) pregnant or lactating females; and (6) subjects taking immunosuppressant medications. All patients were required to sign an informed consent in order to participate in the study.

To establish controls, all patients received a dental examination, dental prophylaxis when indicated, and oral hygiene instructions to address habits related to disease etiology and to demonstrate effective plaque control prior to surgical procedures. Teeth in the quadrant involved in the study received periapical radiographs, if not previously available in the chart, and were tested for vitality. Within each patient, the quadrant with qualifying recession was randomly assigned to either the experimental or control group using a randomization table.

Random numbers were assigned to each patient as they were recruited for the study. The patients assigned to the test group were treated with OrACELL™ on roots of the corresponding qualifying sites. The patients assigned to the control group were treated with an autogenous connective tissue graft on roots of the corresponding control sites. Factors such as oral hygiene, compliance, and varied healing responses were therefore controlled by randomization.

### ***Clinical Parameters***

The following clinical parameters were evaluated: vertical recession (VR) measured as the distance from the cemento-enamel junction (CEJ) to the free gingival margin (FGM) in mm; horizontal recession (HR) measured at the CEJ in mm; probing depth (PD) on midfacial aspect measured as the distance from the FGM to the bottom of the sulcus in mm; clinical attachment level (CAL) measured as the distance from the CEJ

to the bottom of the sulcus in mm; presence or absence of bleeding on probing (BOP) on midfacial aspect; papillary height (PH), defined as the distance from the tip of the papilla to the base of the papilla at level of the CEJ; papillary width (PW) measured at the base of the papilla at CEJ level for both the mesial and distal papilla adjacent to the recession; and the width of the keratinized tissue (KT) from the FGM to the mucogingival junction in mm.

The distance from the CEJ to bone at midfacial aspect was measured after flap reflection. All measurements were made with a UNC periodontal probe (15mm). Clinical parameters VR, HR, PD, CAL, BOP, PH, PW, and KT were recorded prior to surgery (baseline) and again at 3 and 6 months post operatively. When the CEJ was obliterated by a non-carious cervical lesion, the most coronal aspect of a non-carious cervical lesion served as a reference point for VR measurements. All parameters, including Miller recession classification, were evaluated by 2 blinded Board Certified Periodontists.

### ***Surgical Procedure***

The surgical procedure was identical for both test and control groups with the only difference being the graft material. On the day of surgery, the exposed root surfaces were thoroughly root planed by means of curettes to ensure a smooth biocompatible surface. The sites were rinsed with 0.9% normal saline solution. The surgical site was anesthetized with (2%) lidocaine HCl with 1:100,000 epinephrine. The surgery began with incisions described by Zuchelli, which consist of making “horizontal incisions of an envelope flap consisting of oblique submarginal incisions interdentally continuous with intrasulcular incisions at the recession defects” (64). During coronal advancement, each

surgical papilla was rotated towards the ends of the flap (papilla mesial to the midline of the flap rotate in the mesial-coronal direction, while papilla distal to the midline of the flap rotate in the distal-coronal direction) (64). Finally, the flapped papillae were positioned over the de-epithelialized intact anatomic papillae. Full thickness flaps were reflected approximately 3mm apical to the alveolar bone crest at each defect site. Starting at the coronal aspect, a split-thickness flap dissection was performed to facilitate adequate mobility for the coronal advancement of the flap. The anatomic papillae were then de-epithelialized to ensure a good vascular connective tissue bed. The OrACELL™ graft was then prepared. After opening of the outer packaging pouch, the inner pouch was opened and the OrACELL™ graft was removed aseptically. Using forceps, the tissue was removed and trimmed to fit in the recession area. The graft was adjusted to completely cover the defect and superior graft margin positioned at the CEJ, while the inferior and lateral borders of the graft were extended at least 3 mm beyond the osseous defect margins. OrACELL™ was placed against the root surface and sutured using the double sling suture technique with 5-0 chromic gut suture. The flap was coronally positioned to cover the entire allograft and also sutured using the double sling suture technique with additional interrupted 6-0 Prolene® sutures, as needed, to ideally position the papillae. Flap closure was accomplished with 6-0 Prolene® sutures.

The control group underwent the same surgical procedure as the test, substituting OrACELL™ for an autogenous connective tissue graft (CTG). In the control group, the CTG was harvested from the palate in a surgical approach described by Langer (45). The CTG was thinned to 1.5-2mm prior to placement at the recipient bed.



### ***Post-Surgical Care***

After the procedure, patients were prescribed 500 mg amoxicillin, three times daily for 7 days, or 300 mg clindamycin, three times daily for 7 days if the patient was allergic to penicillin. In addition, 50mg Tramadol was prescribed for post-operative analgesia. Ice pack application was used immediately after surgery on an intermittent basis for the first 3 to 4 hours at both control and test sites. All patients were advised to discontinue mechanical oral hygiene measures in the surgical areas for 2 weeks and avoid trauma to the surgical sites. A liquid diet was recommended for the first 48 hours followed by a soft diet for the next 2 weeks. Chlorhexidine gluconate 0.12% rinse was prescribed for 2 weeks with instruction to rinse only on a daily basis. Gentle tooth brushing was resumed at 2 weeks using a roll technique until the 1-month follow-up appointment. Professional plaque control was performed at the 1 week, 1 month, 3 month, and 6 month recall appointments.

## CHAPTER III

### RESULTS

Twenty-four patients (15 females, 9 males, aged 26 to 78 years; mean age: 50.5 years) had one defect treated each, for a total of 24 defects. All twenty-four patients that enrolled in the study completed the study, with no dropouts. There were 23 Miller Class III defects and one Miller Class I defect which consisted of 12 incisors, 4 canines, and 8 premolars. Eleven sites were treated with CTG (control) while thirteen sites were treated with OrACELL™ (test).

Results were tabulated and analyzed as described above using SAS 9.3, utilizing univariate ANOVA. In a longitudinal study using this analysis, the purpose is to test for outcome as a function of time, and to determine if there is a significant difference between treatment groups. This analysis of individual variables between treatment groups was completed with an  $\alpha = 0.05$  and interaction was tested between the variable of interest and time (baseline, 3 months, 6 months).

$H_0$ : There is no interaction effect for the variable in consideration.

$H_A$ : There is an interaction effect for the variable in consideration.

If this test rejects the null (i.e.  $p < 0.05$  in the above hypothesis test), then the test for treatment effect is as follows:

$H_0$ : There is a treatment effect for the variable in consideration.

$H_A$ : There is no treatment effect for the variable in consideration.

and for time:

$H_0$ : There is no time effect for the variable in consideration.

$H_A$ : There is a time effect the variable in consideration.

Therefore, when  $p < 0.05$  then the null hypothesis is rejected and the difference between the test and control groups is significant. Baseline, 3 month, and 6 month clinical measurements for test and control groups are summarized in Table 1. At baseline there was no statistically significant difference in parameters between the test and control groups.

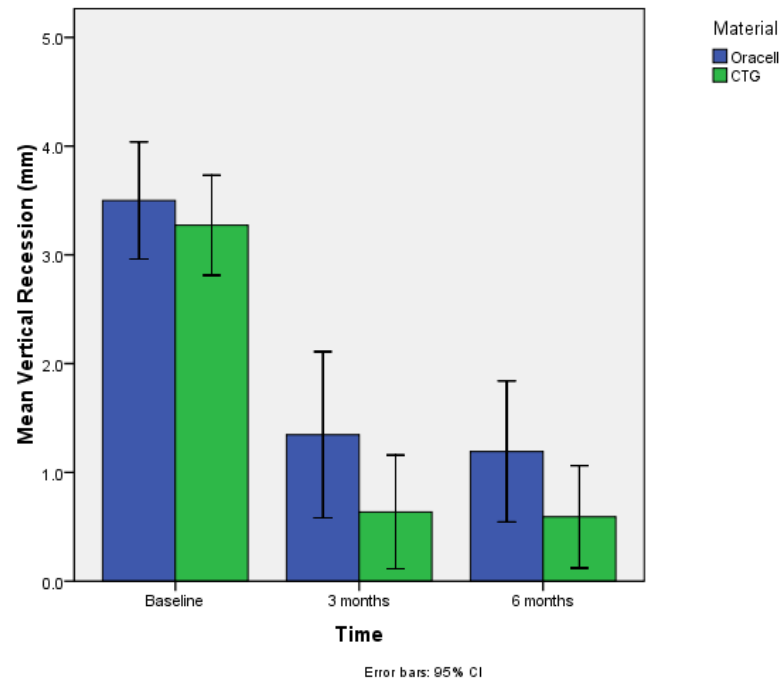
	<b>Baseline (mm)</b>	<b>Baseline (mm)</b>	<b>3 month (mm)</b>	<b>3 month (mm)</b>	<b>6 month (mm)</b>	<b>6 month (mm)</b>
	<b>CTG</b>	<b>OrACELL™</b>	<b>CTG</b>	<b>OrACELL™</b>	<b>CTG</b>	<b>OrACELL™</b>
<b>VR</b>	3.27±0.68	3.50±0.89	0.64±0.78	1.35±1.26	0.59±0.70	1.19±1.07
<b>HR</b>	3.50±1.02	3.35±0.66	1.91±1.77	2.08±1.55	1.86±1.61	2.31±1.44
<b>PD</b>	1.59±0.58	1.23±0.39	1.23±0.68	1.39±0.55	1.23±0.52	1.31±0.43
<b>CAL</b>	4.86±0.74	4.73±0.90	1.86±0.87	2.73±1.25	1.91±1.00	2.42±1.17
<b>KT</b>	1.96±1.80	2.04±0.95	2.23±1.15	2.58±1.47	2.55±1.62	2.50±1.58
<b>PHM</b>	3.36±1.05	3.46±0.83	2.86±0.67	3.23±0.86	2.77±0.47	3.27±0.78
<b>PHD</b>	3.32±1.03	3.58±0.93	2.82±0.75	3.35±0.75	2.86±0.64	3.31±0.72
<b>PWM</b>	3.68±0.84	3.23±0.86	3.23±0.75	2.69±0.72	3.00±0.71	2.85±0.59
<b>PWD</b>	3.36±0.71	3.23±0.83	3.05±0.79	3.00±1.00	2.82±0.75	2.96±0.78

**Table 3-1:** Mean ( $\pm$  SD) of variables at baseline, 3-, and 6-months

### Vertical Recession

Progression of VR in both groups over time is summarized in Figure 1. In general, both CTG and OrACELL™ had significant improvement in VR when compared to baseline ( $p=0.001$ ). Baseline VR data showed no significant difference between groups (3.27±0.68 mm for CTG and 3.50±0.89 mm for OrACELL™). At 3 months, remaining VR decreased in both groups (0.64±0.78 mm for CTG and 1.35±0.1.26 mm for OrACELL™). At 6 months, in both the CTG and OrACELL™ treated sites, VR continued to decrease to 0.59±0.70 mm and 1.19±1.07 mm, respectively. At 6 months,

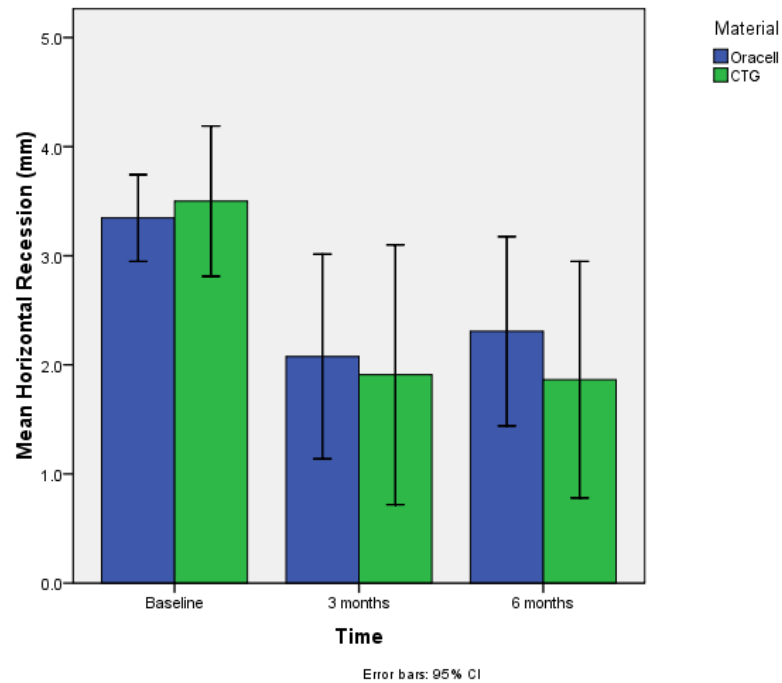
CTG treated sites showed greater improvement in VR compared to OrACELL™, but the difference was not statistically significant. When time and treatment groups were analyzed simultaneously the significance was  $p=0.650$ .



**Figure 3-1:** Mean vertical recession at baseline, 3-, and 6-months

### Horizontal Recession

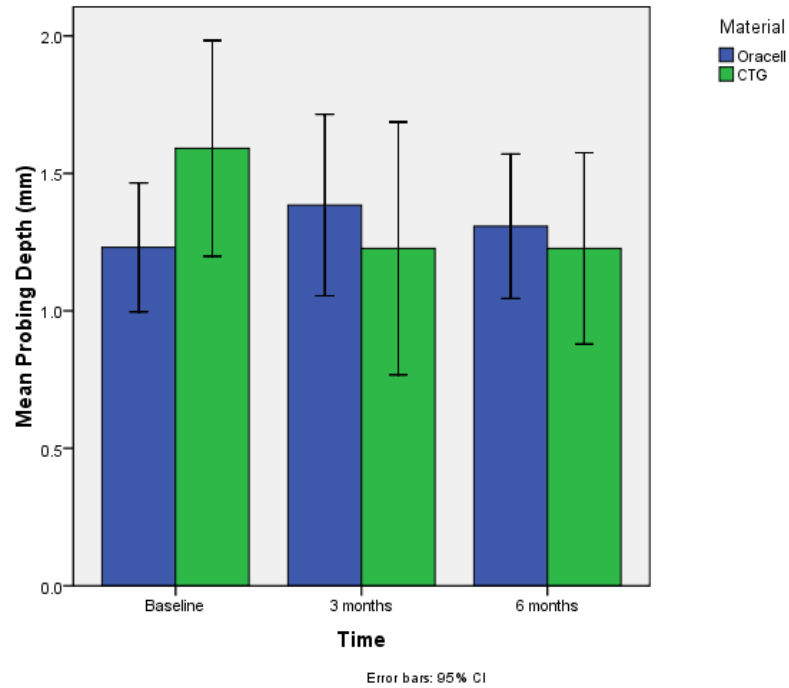
There were no significant differences over time between groups in HR ( $p=0.758$ ), but there was a significant decrease in HR in both groups ( $p=0.001$ ). Baseline mean HR was  $3.50 \pm 1.02$  mm in CTG and  $3.35 \pm 0.66$  mm in OrACELL™. At 3 months, HR decreased to  $1.91 \pm 1.77$  mm in CTG and  $2.08 \pm 1.55$  mm in OrACELL™. At 6 months, HR decreased to  $1.86 \pm 1.61$  mm in CTG and increased to  $2.31 \pm 1.44$  mm in OrACELL™. The results are summarized in Figure 2.



**Figure 3-2:** Mean horizontal recession at baseline, 3-, and 6-months

### Probing Depth

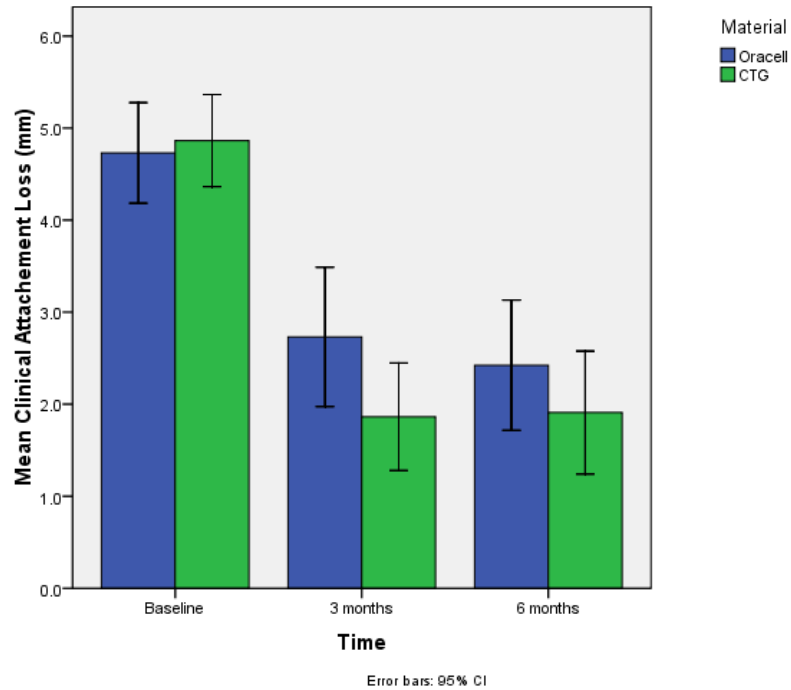
At baseline, the PD for CTG and OrACELL™ were  $1.59 \pm 0.58$  mm and  $1.23 \pm 0.39$  mm, respectively. At 3 months, PD decreased in CTG to  $1.23 \pm 0.68$  mm and increased in OrACELL™ to  $1.39 \pm 0.55$  mm. At 6 months, PD remained unchanged at  $1.23 \pm 0.52$  mm for CTG and decreased to  $1.31 \pm 0.43$  mm for OrACELL™. It should be noted that CTG maintained a slight reduction in PD compared to OrACELL™ at both 3 months and 6 months; however, all intergroup differences did not reach statistical significance. Figure 3 provides a summary of this data.



**Figure 3-3:** Mean probing depth at baseline, 3-, and 6-months

### Clinical Attachment Level

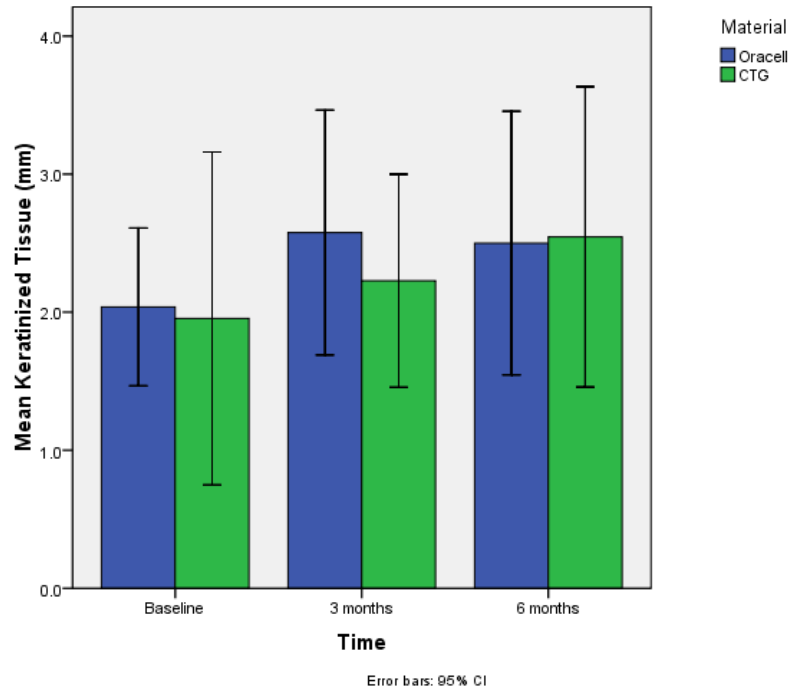
Progression of CAL in both groups over time is summarized in Figure 4. CAL improved significantly for both groups between baseline and 6 months ( $p < 0.001$ ). The mean CAL at baseline for CTG was  $4.86 \pm 0.74$  mm and  $4.73 \pm 0.90$  mm for OrACELL™. At 3 months, CAL decreased to  $1.86 \pm 0.87$  mm and  $2.73 \pm 1.25$  mm for CTG and OrACELL™, respectively. At 6 months, CAL increased in CTG to  $1.91 \pm 1.00$  mm and decreased in OrACELL™ to  $2.42 \pm 1.17$  mm. Difference in mean CAL between CTG and OrACELL™ was statistically significant at 3 months only. When treatment group and time were analyzed at 6 months and globally, no significant interaction or difference was found ( $p=0.234$ ).



**Figure 3-4:** Mean clinical attachment loss at baseline, 3-, and 6-months

### Keratinized Tissue

At baseline, mean KT was  $1.96 \pm 1.80$  mm and  $2.04 \pm 0.95$  mm for CTG and OrACELL™, respectively. At 3 months, KT increased to  $2.23 \pm 1.15$  mm for CTG and  $2.58 \pm 1.47$  mm for OrACELL™. At 6 months, mean KT increased slightly to  $2.55 \pm 1.92$  mm for CTG, and decreased slightly to  $2.50 \pm 1.58$  mm for OrACELL™. Both groups had a greater amount of KT gain as a result of the procedure (approximately 0.50 mm). However, changes in KT over time were not statistically significant ( $p=0.426$ ) nor were intergroup differences over time ( $p=0.891$ ). Progression of KT in both groups over time is summarized in Figure 5.



**Figure 3-5:** Mean keratinized tissue at baseline, 3-, and 6-months

### Papillary Measurements

Papillary measurements are summarized on Table 1. Generally, PHM, PHD, PWM, PWD decreased in both groups over time. For all four groups [PHM ( $p=0.681$ ), PHD ( $p=0.843$ ), PWM ( $p=0.655$ ), PWD ( $p=0.839$ )], the difference between baseline and 6 months did not reach statistical significant between groups. For PHM ( $p=0.175$ ), PHD ( $p=0.212$ ), and PWD ( $p=0.225$ ), the difference between baseline and 6 months did not reach statistical significance over time. The PWM was  $3.68 \pm 0.84$  mm,  $3.23 \pm 0.75$  mm, and  $3.00 \pm 0.71$  mm for CTG at baseline, 3 months, and 6 months, respectively. Furthermore, the PWM was  $3.23 \pm 0.86$  mm,  $2.69 \pm 0.72$  mm, and  $2.85 \pm 0.69$  mm for OrACELL™ at baseline, 3 months, and 6 months, respectively. The decrease in PWM



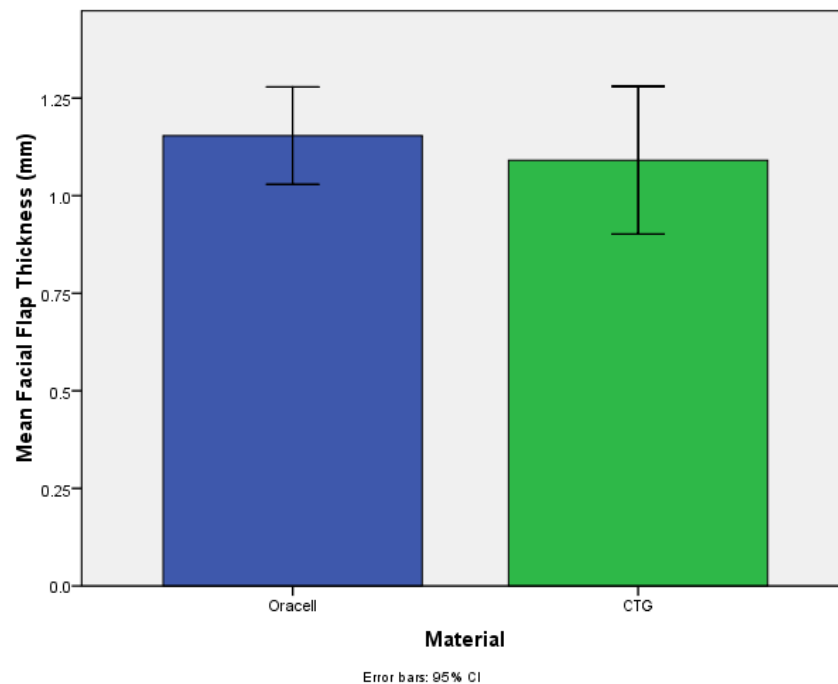
was found to be statistically significant over time ( $p=0.028$ ). Lastly, the difference between groups was not significant at any time point for PHM, PHD, PWD, and PWM.

### Bleeding on Probing

BOP was recorded with ordinal data and was therefore evaluated with a Chi-Square Test. There were no significant differences over time between groups in BOP ( $p=0.795$ ). For CTG, BOP occurred 27.3% at baseline, 18.2% at 3 months, and 9.1% at 6 months. For OrACELL™, BOP occurred 15.4% at baseline, 7.7% at 3 months, and then increased back to 15.4% of sites at 6 months.

### Flap Thickness

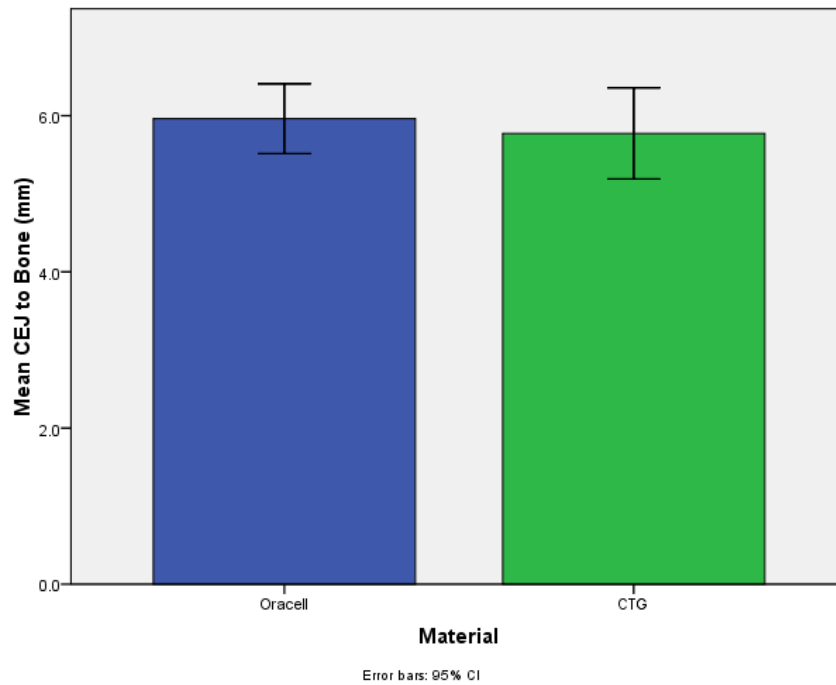
Mean FT was  $1.13 \pm 0.37$  mm for all treated sites. For CTG the mean FT was  $1.09 \pm 0.44$  mm and OrACELL™ the mean FT was  $1.15 \pm 0.32$  mm and is demonstrated in Figure 6. There was no difference in FT between groups.



**Figure 3-6:** Mean facial flap thickness

### Alveolar Crest to CEJ

Mean BH was  $5.88 \pm 1.21$  mm for all treated sites. For CTG the mean BH was  $5.77 \pm 1.35$  mm and OrACELL™ the mean BH was  $5.96 \pm 1.13$  mm and is demonstrated in Figure 7. There was no difference in BH between groups.



**Figure 3-7:** Mean distance from CEJ to bone (BH)

## CHAPTER IV

### DISCUSSION

This study set out to aid in the search for a material that can perform similarly clinically in providing root coverage to that of the gold standard, CTG. Decellularized dermis/OrACELL™, unlike ADM and ECM, has little current literature regarding its efficacy as a CTG substitute. The target for many clinicians is to find a material that decreases the post-operative morbidity of requiring a palatal harvest site yet still maintains a similar effectiveness for root coverage as CTG. Decellularized dermis, like ADM and ECM, reduces post-operative morbidity and surgical time since a second palatal surgical site is not required. Decellularized dermis also allows for significantly more sites to be treated in one surgery, as there is a limitless supply of graft material.

The current reporting trends of root coverage can be misleading and deceiving, according to Greenwell *et al*, because only the amount of soft tissue covering the original defect tends to be calculated (81). For example, a tooth with 4 mm of VR is treated and the result is the VR is decreased to 2 mm. Following current trends, this finding would be reported as 50% of root coverage being achieved. According to Greenwell *et al*, this method doesn't appropriately explain the measurement of root coverage and should rather be referred to as "defect coverage calculated" (81). Greenwell goes on to suggest it be calculated as follows:

$$\% \text{ Defect Coverage} = (1 - \text{VR}_t / \text{VR}_0) \times 100$$

$\text{VR}_0$  = baseline VR and  $\text{VR}_t$  = VR at time t.

Greenwell *et al* recommends labeling root coverage as a function of VR at baseline and after treatment over root length, i.e. 5 mm of VR on a 15 mm length root would have 66% root coverage. Subsequently, the actual root length of every tooth that is treated is often unknown. Therefore, Greenwell *et al* recommends a generally accepted universal root length of 13.63 mm for standardization of calculation (113):

$$\% \text{ Root Coverage} = 100 \times (13.63 - \text{VR}_t) / 13.63$$

Table A-1 provides a summary of percent root coverage in test and control groups over time according to criteria set by Greenwell *et al*. See Table A-2 for a summary of percent defect coverage according to criteria set by Greenwell *et al*.

Greenwell *et al* positions that defect elimination is successfully achieved when 95 to 100% mean root coverage is obtained (81). At 6 months, 95.66% mean root coverage was achieved for CTG and 91.25% mean root coverage for OrACELL™, as seen in Table 2. At 6 months the defect coverage was 81.94% and 65.94% for CTG and OrACELL™, respectively, which is illustrated in Table 3. These findings are likely explained by the inclusion of Miller Class III defects in 23 of the 24 subjects. Miller explained that complete root coverage wasn't realistic in Class III defects due to interproximal bone loss, extrusion, or malpositioning (31). The surrounding blood supply for a graft in a Miller Class III defect is often apical to the CEJ and therefore both graft and overall tissue height cannot be maintained at the CEJ.

It was noted that the authors often found at sites included in this study teeth that were misaligned outside the alveolus. The baseline average distance from the CEJ-to-

bone was 5.87 mm, and it's plausible that malpositioning lead to an absence of buccal alveolar housing, which subsequently initiated gingival recession at the site. The vascular innervation to the site from the periodontal ligament, alveolar bone, and suprapariosteal vessels is often compromised in these malpositioned Miller Class III defects.

Conversely, a tooth that is rotated but not outside the alveolar housing doesn't necessarily threaten potential root coverage because it usually has superior periodontal ligament- and suprapariosteal-blood supplies.

Marini *et al* evaluated gingival recession in 380 adult subjects and found that 3,526 teeth had a total 6,123 surfaces with recession. This ultimately equated to 89% of subjects exhibiting at least one tooth surface with gingival recession (114). Furthermore, Marini *et al* found that the majority of these defects were 59% Miller Class I and 33% Class III defects (114). Twenty-three of the 24 sites analyzed in this study were Miller Class III recession defects due to interproximal bone loss and tooth malpositioning. The authors included Miller Class III defects because of the significant prevalence of these defects among individuals with gingival recession. The authors also found in this study that Miller Class III defects treated with OrACELL™ failed to demonstrate a significant difference in remaining VR and CAL when compared to defects treated with CTG over a 6-month follow-up. There was a statistically significant difference in CAL at 3 months, but the difference was no longer found to be statistically significant at 6 months. Gingival recession studies commonly limit recession defect inclusion to only Miller Class I and II defects because there is increased treatment predictability with Miller Class I and II defects. With Miller Class III defects being less predictable, they may perhaps serve as a better gauge of material efficacy. Historically, CTG would serve as the material of choice

for treatment of Miller Class III defects due to its predictability and status as the gold standard. However, this study demonstrates that OrACELL™ was as effective as CTG in the treatment of Miller Class III defects.

Similar findings to this current study have been reported in other studies that included Miller Class III recession defects. Few studies have reported on the use of decellularized dermis for root coverage but numerous investigators have reported on ADM. Barker compared the differences in root coverage of Miller Class I and III defects, using two different ADM products (AlloDerm® and Puros® Dermis). This study reported mean defect coverage of 81.4% and 83.4% using the two products (97). Mean root coverage was 95.2% for AlloDerm® and 95.1% for Puros® Dermis when using the Greenwell *et al* criteria for root coverage. Another study by Shin treating 42 Miller Class I and 40 Miller Class III defects in 14 patients reported mean defect coverage of 73.4% for ADM and 79.4% for ADM plus enamel matrix derivative (EMD) (115). When using the Greenwell *et al* criteria for root coverage, the mean was 93.1% for ADM and 94.1% for ADM plus EMD. Miller Class I, II, and III defects were examined in a study by Carney using ADM with and without growth factors (GEM21™). Carney reported average defect coverage of 76.7% in the group without addition of growth factors and 69% for the group with growth factors (116). When analyzed separately, Miller Class III defects demonstrated mean defect coverage of 60.8% and 51.5% for ADM and ADM plus rhPDGF, respectively. When using the Greenwell *et al* criteria for root coverage, mean root coverage was 94.4% for ADM and 95.2% for ADM plus rhPDGF.

An interesting finding from this study was the increase in keratinized tissue for both the CTG and OrACELL™ groups. There was no statistically significant difference

between groups at baseline, 3 months, or 6 months but there was a gradual incremental increase from baseline to 6 months. KT for CTG improved from an average of  $1.96 \pm 1.80$  mm at baseline to  $2.55 \pm 1.62$  mm at 6 months, while OrACELL™ enhanced from  $2.04 \pm 0.95$  mm to  $2.50 \pm 1.58$  mm. While neither of these findings is statistically significant, the authors believe they may be clinically significant. This investigation is not the first to report an improvement in KT after a root coverage procedure. Barros reported a gain of KT of 1mm at 6 months after ADM procedures for root coverage in Miller Class I and II defects (117). Also looking at Class I and II defects with ADM for root coverage, Aichelmann-Reidy reported a gain in KT of 1.2mm at 6 months (118). According to Karring *et al*, the genotype of underlying connective tissue determines the character of the overlying epithelium (119). The use of decellularized dermis produced small but similar increases in KT compared to CTG, which undermines the mechanism described by Karring *et al*. This leads the authors to speculate that the increase in KT from decellularized dermis is potentially a result of measuring error or perhaps a signaling process that is not currently well understood.

This randomized clinical trial failed to find any statistical differences in VR at any time point in the treatment of gingival recession defects. While there was a small but significant difference in CAL between groups at 3 months, no statistically significant difference remained regarding CAL after 6 months of healing. Evidence from this study supports the use of OrACELL™ in root coverage procedures in Miller Class I, II, and III defects. Conclusions drawn from this study pertain solely to OrACELL™ as it was the only decellularized dermis product tested. Future studies should include long-term follow-up and larger sample populations. Additionally, histologic comparison of CTG

and OrACELL™ tissue biopsies should be evaluated for differences in root attachment and keratinization.



## CHAPTER V

### CONCLUSION

This randomized clinical trial failed to find any statistical differences in VR at any time point in the treatment of gingival recession defects between the test and control groups. While there was a small but significant difference in CAL between groups at 3 months, no statistically significant difference remained regarding CAL after 6 months of healing. Evidence from this study supports the use of OrACELL™ in root coverage procedures in Miller Class I, II, and III defects. Conclusions drawn from this study pertain solely to OrACELL™ as it was the only decellularized dermis product tested. Future studies should include long-term follow-up and larger sample populations. Additionally, histologic comparison of CTG and OrACELL™ tissue biopsies should be evaluated for differences in root attachment and keratinization.

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## APPENDIX

### TABLES

**Table A-1**

Percent Root Coverage at Baseline and at 3 and 6 Months\*

	Baseline	Baseline	3 Months	3 Months	6 Months	6 Months
Material	CTG	OrACELL	CTG	OrACELL	CTG	OrACELL
% Root Coverage	75.98%	74.32%	95.33%	90.12%	95.66%	91.25%

\* % Root Coverage =  $100 \times (13.63 - VR_t) / 13.63$  ;  $VR_t$  = VR at time t. Greenwell *et al* criteria.

**Table A-2**

Percent Defect Coverage at 3 and 6 Months\*

	3 Months	3 Months	6 Months	6 Months
Material	CTG	OrACELL	CTG	OrACELL
% Defect Coverage	80.56%	61.54%	81.94%	65.94%

\* % Defect Coverage =  $(1 - VR_t / VR_0) \times 100$  ;  $VR_0$  = baseline VR,  $VR_t$  = VR at time t.



**Figure A-1**

OrACELL™ surgical procedure and 6 month follow up photos. Starting from Top Left: Initial, OrACELL™ placed and sutured, Flap closure, 6 month follow up.